Application of Synchrotron Radiation-Based Fourier Transform Infrared Spectromicroscopy to the Investigation of Bacterial Attachment to Distinct Mineral Phases Within a Mineralogically Heterogeneous Geologic Substrata

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INTRODUCTION

Trichloroethylene (TCE) is a major contaminant in the Snake River Plain Aquifer below the Test Area North (TAN) facility of the Idaho National Engineering and Environmental Laboratory (INEEL) in southeastern Idaho. The dominant hydrogeology of the aquifer is fractured Pleistocene and Holocene olivine basalt with intercalated sedimentary beds. Microbial community profiles of the groundwater from wells at the TAN facility indicate the presence of isolates similar to *Burkholderia cepacia* G4, a microorganism capable of cometabolizing TCE via the toluene 2-monooxygenase catabolic pathway. Extensive studies of the microbial ecology of groundwater systems indicate that portions of the microbial community will attach to surficial material within the aquifer. Understanding the organic-inorganic interactions that occur at the bacterial-mineral interface is critical to the design and implementation of possible in situ bioremediation technologies. The objective of this study is to determine if a pure culture of TCE-cometabolizing bacteria exhibit preferential attachment to specific mineral phases within a heterogeneous geologic substratum and if so, to determine if attachment to specific mineral phases affects the microorganisms' capability of producing the requisite catabolic enzymes.

EXPERIMENT

Synchrotron radiation-based (SR) Fourier transform infrared (FTIR) spectromicroscopy was used to study bacterial attachment to distinct mineral phases within a heterogeneous basalt. The bacterial-mineral interface spectra were collected using the 1.4.3 Beamline experimental endstation at the Lawrence Berkeley National Laboratory (LBNL) Advanced Light Source (ALS). FTIR spectra were recorded in the 4000-650 cm^{-1} infrared region at a spectral resolution of 4 cm^{-1} and microscope-focused experimental spot size of 10 μm. Basalt specimens were spectrally characterized prior to, and after, inoculation with a pure culture of *Burkholderia cepacia* G4. Spectra of the distinct mineral phases in the basalt were compared to spectra obtained for individual mineral standards. Preliminary experiments involved culturing bacteria on polished aluminum slides for FTIR analysis. The highly reflective aluminum surface maximized the spectral resolution and aided in obtaining reference spectra of *Burkholderia cepacia* G4 cells. Petrographic thin sections of basalt were prepared from core samples obtained from one of the wells at the INEEL TAN facility. The thin sections were petrographically characterized by polarized light microscopy. The olivine basalts contain calcic

plagioclase, clinopyroxene, olivine, ilmenite, and accessory apatite. The thin sections were then exposed to a pure culture of bacteria. The thin sections were sterilized prior to inoculation by sonication in distilled H_2O, followed by exposure to UV light for 20 minutes per side. The thin sections were then immersed in a pure culture of bacteria for 24 hours.

RESULTS

SR-FTIR microspectroscopic mapping was conducted an aluminum slide exposed to a pure culture of *Burkholderia cepacia* G4 for 16 hours. Figure 1 is a 3D map showing the localization of the bacteria on the aluminum slide within a 100 X 150 µm area, with 10 µm step widths in both the X and Y directions. Sixteen scans were taken at each location with a resolution of 4 cm^{-1}. SR-FTIR microspectroscopic mapping was also conducted on an area of an inoculated thin section. Figure 2 illustrates the different mineral phases of calcic plagioclase, augite, and olivine within the basalt thin section and the individual spectra extracted from the map data illustrate the peaks associated with bacterial cell wall functional groups. The spectral characterizations of both the aluminum slide and basalt specimens after inoculation revealed absorption peaks near 1740, 1650, and 1550 cm^{-1}, which correspond to the phospholipids in the outer membrane of Gram-negative bacteria, protein Amide I, and protein Amide II, respectively. These absorption bands, indicative of bacterial cell walls, were consistent with previously published literature on infrared spectroscopy of biomolecules. Preliminary results indicate that SR FTIR spectromicroscopy can be a valuable tool in evaluating the contribution of distinct mineral phases within heterogeneous geologic substrata to subsurface microbiological environments. Future SR-FTIR work will include analysis of toluene and TCE contaminated basalt specimens exposed to pure cultures of *Burkholderia cepacia* G4 to determine if attachment to various mineral phases within the basalt affects the microorganisms' ability to produce the requisite catabolic enzymes for TCE cometabolization.

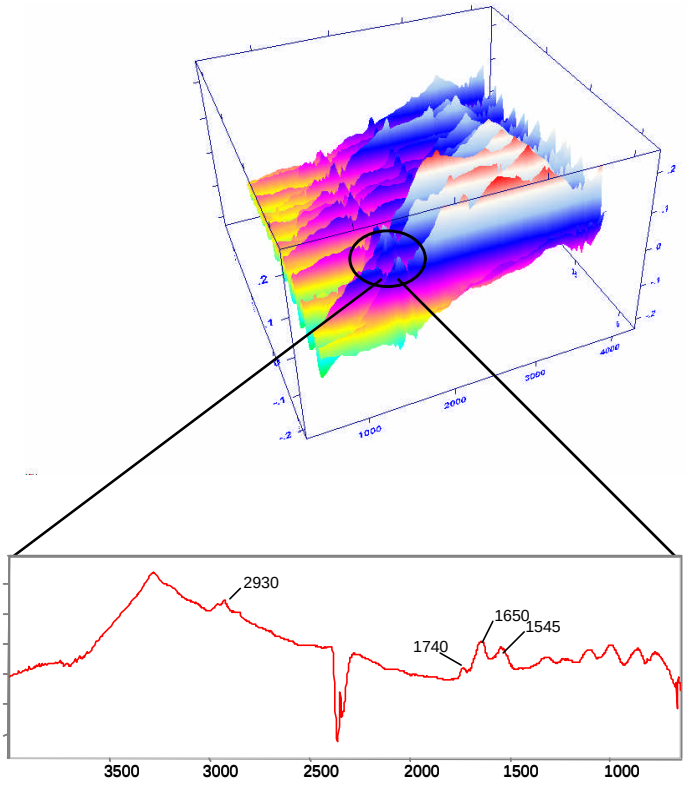

Figure 1. Individual spectra extracted from map data indicating localization of bacteria attached on an aluminum slide

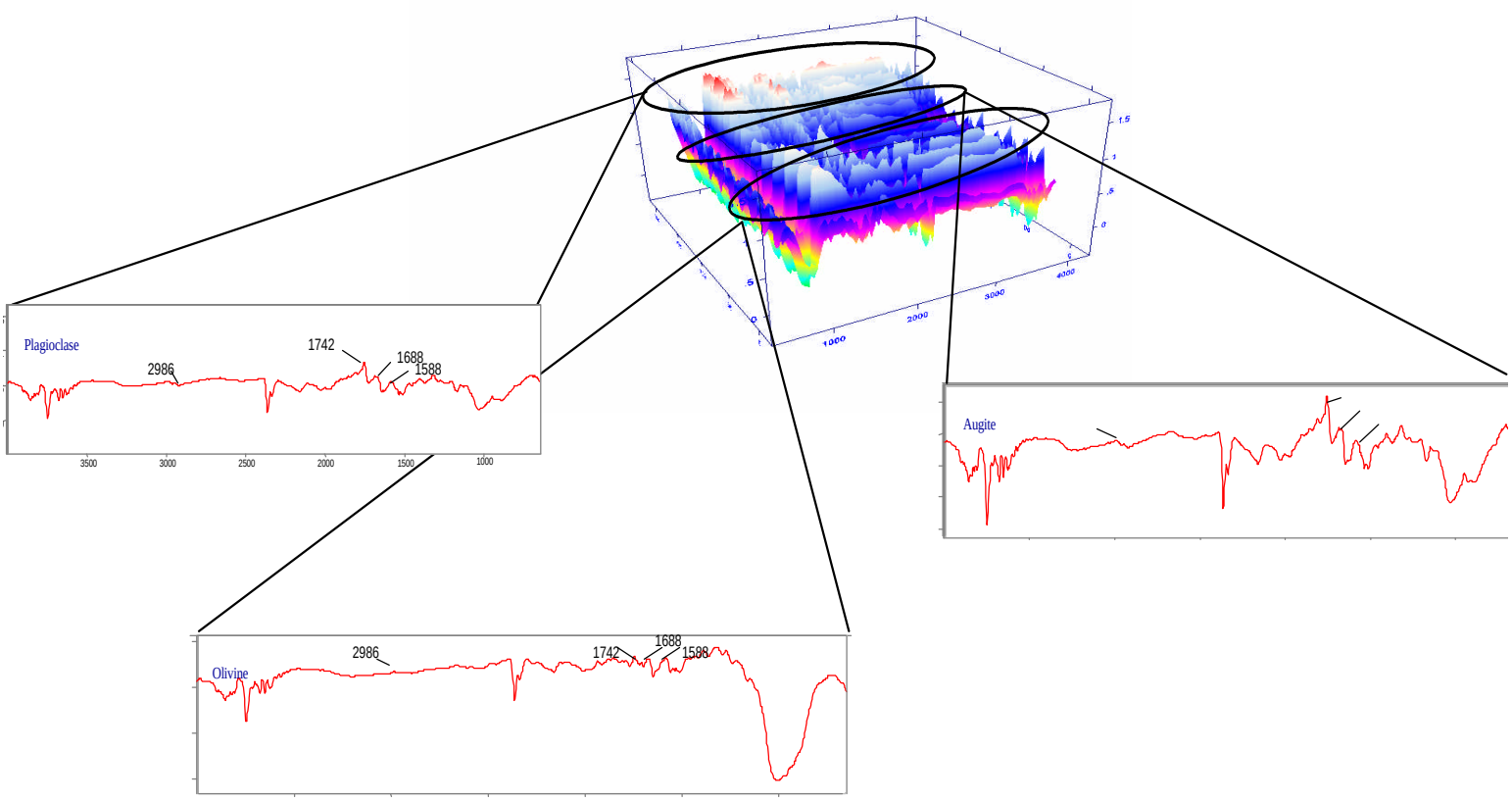

Figure 2. Map is 105 X 175 µm, with a 10 µm step distance in both the X and Y directions. Sixteen scans were taken at each location with a resolution of 4^{-1} cm. The 3D area map above shows the different mineral phases of calcic plagioclase, augite, and olivine within the basalt thin section. The individual spectra extracted from the map data illustrate the peaks associated with bacterial cell wall functional groups. The shifting of the peaks is believed to be due to the thickness of the bacterial biofilm.

This work was supported by the U.S. Department of Energy, Office of Environmental Management at the Idaho National Engineering and Environmental Laboratory operated under contract DE-AC-07-99ID13727 by Bechtel BWXT Idaho, LLC.

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